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1. Your reference

40221(2)/JMD

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3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

PEPTIDE THERAPEUTICS LTD.
Peterhouse Technology Park,
100, Fulbourn Road,
Cambridge CB1 9PT.

Patents ADP number *(if you know it)*

6428007003

If the applicant is a corporate body, give the country/state of incorporation

United Kingdom

4. Title of the invention

Therapeutic Antibody, Immunogenic Compositions and Uses

5. Full name, address and postcode in the United Kingdom to which all correspondence relating to this form and translation should be sent

Reddie & Grose
16 Theobalds Road
LONDON
WC1X 8PL

Patents ADP number *(if you know it)*

91001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application *(If you know it)*	Date of filing *(day/month/year)*

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Number of earlier application	Date of filing *(day/month/year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

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Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	NO
Request for preliminary examination and search *(Patents Form 9/77)*	NO
Request for substantive examination *(Patents Form 10/77)*	NO
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature Reddie e Grose Date 7 May 1999

12. Name and daytime telephone number of person to contact in the United Kingdom: J M DAVIES 01223-360350

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Therapeutic Antibody, Immunogenic Compositions and Uses

The present invention relates to novel therapeutic compositions containing an anti-IgE antibody, and its use in the preparation of medicaments for the treatment or prophylaxis of IgE-mediated allergies. Where legally permissible, the invention also provides a method of treatment/prophylaxis using such compositions of antibodies.

In a second aspect, the invention relates to use of the antibody for determination of therapeutically "useful" sequences within the IgE molecule and mimotopes thereof, which may be used to form the basis of an anti-IgE vaccine for treatment/prophylaxis of allergic disease.

In a third aspect, the invention relates to a novel epitope and its use in the preparation of medicaments for the treatment or prophylaxis of IgE-mediated allergies.

The term "antibody" herein is used to refer to a molecule having a useful antigen binding specificity. Those skilled in the art will readily appreciate that this term may also cover polypeptides which are fragments of or derivatives of antibodies yet which can show the same or a closely similar functionality. Such antibody fragments or derivatives are intended to be encompassed by the term antibody as used herein.

Various classes of antibodies are known which may generically be called "anti-IgE antibodies". These may recognise one or more regions of the IgE molecule. For example, monoclonal antibodies are known which bind to the human IgE heavy chain binding site for mast cells (WO 93/04173). More specifically, antibodies are known which bind to the IgE Fc domain (WO 89/04834 and WO 90/15878).

Depending on their particular nature, anti-IgE antibodies may bind IgE either in its receptor-bound state or non-receptor-bound (i.e. free solution or bound to a solid phase).

Known Anti-IgE antibodies are generally anaphylactogenic, i.e. they cause triggering of basophils or mast cells by cross-linking of IgE bound to its high affinity receptor (FcεRI), resulting in degranulation and release of histamine. Consequently such antibodies are therapeutically useless and indeed are likely to be positively dangerous if administered to a patient.

Nonetheless, such antibodies are commercially available and have been sold as IgE detection agents for use in techniques such as Western blotting and immunohistochemistry. Those skilled in the art fully understand that in this field the mere existence of anti-IgE activity in an antibody does not imply any useful therapeutic or prophylactic properties.

Much work has been carried out by those skilled in the art to identify specific anti-IgE antibodies which do have some beneficial effects against IgE-mediated allergic reaction (WO 90/15878, WO 89/04834, WO 93/05810). Attempts have also been made to identify epitopes recognised by useful antibodies, to create peptide mimetics of such epitopes and to use those as immunogens to produce anti-IgE antibodies. Based on the present state of knowledge in this area, and despite enormous scientific interest and endeavour, there is little or no predictability of what characteristics any antibody or epitope may have and whether or not it might have a positive or negative clinical effect on a patient.

It has been found that an anti-IgE antibody, designated PTmAb0005 (available from Sigma Chemicals Catalogue number I6510, clone number GE-1), has the following properties:

(1) It is capable of binding human IgE in its non-receptor-bound state (i.e. in free solution or when bound to a solid phase support e.g. an ELISA plate).

(2) It is capable of binding IgE bound to its high affinity receptor (FcεRI).

(3) It will prevent binding of IgE to the high affinity IgE receptor (FcεRI).

(4) It will not prevent IgE binding to the low affinity IgE receptor (FcεRII).

(5) It inhibits degranulation of human blood basophils following exposure to antigen.

This antibody is potentially useful as a therapeutic/prophylactic agent due to its combination of non-anaphylactogenicity and ability to stabilise basophils.

According to the invention the antibody may be used in the manufacture of a medicament for the treatment/prophylaxis of IgE-mediated immune response such as allergy.

In addition, the antibody can be used to determine sequences within the IgE molecule and mimotopes thereof, which could form the basis of an anti-IgE vaccine, by techniques familiar in the art including, but not limited to, the use of phage display (WO 92/07077).

The antibody may be used in a method of treatment of IgE-mediated immune response such as allergy by administering antibody, fragments thereof, or humanised versions thereof, to a patient as a vaccination to provide passive immune protection against the adverse effects of exposure of the patient to allergen.

The antibody may be used according to the invention against all types of IgE-mediated allergies since the antibody is not dependent on the antigen specificity of the IgE but will react with the total IgE pool of the patient.

Known anti-IgE antibodies may target regions of the human IgE heavy chain, such as sites within the CH2 (WO 93/05810), CH3 (WO 89/04834), or CH4 (WO 90/15878) regions of the epsilon chain (Cε2, Cε3, Cε4 regions). However no overall theory of activity yet appears to have emerged which could predict a subclass having clinically useful properties. Without wishing to be bound by theory, it may be concluded that the unexpected activity found for the antibody useful in the present invention may be due in part or in full to the recognition of a heretofore unexpected site in the IgE Cε2 Fc domain. Although others have reported sequences in this region of the IgE molecule described in WO 93/05810 and WO 98/24808, these sequences are large and of little use for pharmaceuticals. Surprisingly, the anti IgE antibody described in this invention recognises an epitope within the Cε2 domain described by the epitope QVMDVDL (Seq ID No1). This sequence describes the region of the IgE that non-anaphylactogenic anti IgE antibodies such as PtmAb005 will bind to and hence is useful for the treatment of immune responses such as allergy

Other antibodies recognising the same epitope may be identifiable and would be expected to be useful. Such antibodies would constitute a subclass of anti-IgE antibodies.

In view of the foregoing description it will now be clear to those skilled in the art that the above-mentioned antibody, and epitope could provide a useful therapeutic/prophylactic agent. This agent could be administered (e.g. as a vaccination) by routine clinically acceptable means in appropriate dosage forms and dosage regimes to provide patients with relief from or protection against the adverse clinical effects and symptoms of immune reaction to antigen, e.g. in treatment/prophylaxis of allergy.

Similarly, epitopes from the IgE molecule or mimotopes thereof, which have been defined by use of this antibody, may also be used in an active vaccination approach to the treatment of allergy.

Those skilled in the art will recognise that the epitope of the invention when synthesised as a peptide can be used to elicit an immune response in a host animal when conjugated to a suitable carrier. Such suitable carriers include albumins of sera, globulins of sera, thyroglobulins of animals, haemoglobins of animals, haemocyanins of animals, proteins of ascaris, polylysine, polyglutamic acid, lysine-glutamic acid copolymers. In addition other suitable carriers include those such as diphtheria toxoid, tetanus toxoid and the like. Preferred carriers do not induce a clinically undesirable anti-carrier immune response. An example of such a carrier is protein D (described in WO 91/18926).

The covalent coupling of the peptide to the immunogenic carrier can be carried out in a manner well known in the art. Thus, for example, for direct covalent coupling it is possible to utilise a carbodiimide, glutaraldehyde or (N-[γ-maleimidobutyryloxy] succinimide ester. After the coupling reaction the immunogen can easily be isolated and purified by means of a dialysis method, a gel filtration method, a fractionation method etc.

Administration without or with an adjuvant is also envisaged. *Suitable adjuvants are well known in the art (Vaccine Design - The Subunit and Adjuvant Approach, 1995, Pharmaceutical Biotechnology, Volume 6, Eds. Powell, M.F., and Newman, M.J., Plenum Press, New York and London, ISBN 0-306-44867-X).* An example is aluminium hydroxide.

Peptides used in the present invention can be readily synthesised by solid phase procedures well known in the art. Suitable syntheses may be performed by utilising "T-boc" or "F-moc" procedures.

The invention is illustrated by the results shown by data in the following figures, in which:

Figure 1 shows the concentration dependent binding of antibody PTmAb0005 to IgE.

Figure 2 shows the concentration dependent inhibition of IgE binding to an FcεR1α/IgG construct with antibody PTmAb0005 compared to control.

Figure 3 shows the concentration dependent inhibition of IgE binding to clipped ectodomain of FcεRIα-bound directly to plastic plates, by antibody PTmAb0005, compared to control.

Figure 4 shows the lack of inhibition of IgE binding to FcεRII (CD23) by antibody PTmAb0005.

Figure 5 shows the concentration-dependent blocking of histamine release from allergic human blood basophils with antibody PTmAb0005 compared to control.

Antibody Specification

PTmAb0005 is a mouse IgG2b monoclonal antibody.

Assays and Test Methods

IgE binding assay.

Materials

Material	Source (Product Code)
96 well tissue EIA/RIA plates	Costar (3590)
Reservoir trays	Costar (4870)
Phosphate-buffered saline tablets	Sigma (P-4417)
Bovine serum albumin	Sigma (A-2153)
Tween 20	Sigma (P-1379)
o-Phenylenediamine	Sigma (P-8287)
25% v/v sulphuric acid	Not critical
Phosphate-citrate buffer with sodium perborate	Sigma (P-4922)
Human/mouse chimeric IgE	Serotec (MCA333B)
Mouse anti-human IgE mAb (Clone 0277)	Biogenesis (5118-5004)
Sheep anti-mouse IgG-HRP	Serotec (AAC01P)
Sodium carbonate (Analar grade)	BDH (102404H)
Sodium hydrogen carbonate (Analar grade)	BDH (102474V)

Equipment

Equipment	Supplier
Multichannel pipette	Finnpipette or equivalent
MRX ELISA plate reader	Dynex Technologies
DELFIA 1296-026 Platewasher	Wallac

Method

ELISA protocol for the detection of mouse anti-human IgE mAbs.

Coat plates overnight at 4°C with 100 µl/well human IgE diluted to 1 µg/ml in carbonate buffer (1.59 g sodium carbonate and 2.93 g sodium hydrogen carbonate dissolved to 1 litre after adjustment to pH 9.6).

Wash wells three times with 700 µl/wash (PBS/Tween 0.05%).

Block wells with 150 µl of PBS/Tween 0.05%/50 g/l BSA for 2 hours at 37°C.

Add 100 µl/well of anti-human IgE antibody (standard) over the concentration range of $2x10^6$ to 25.6 pg/ml or test supernatant added undiluted or over a range of dilutions, typically up to 1/100, with dilutions prepared in PBS/Tween 0.05%/10 g/l BSA. Incubate the plate for 1 hour at 37°C.

Wash plates as described.

Add 100 µl/well sheep anti-mouse IgG-HRP conjugated antibody diluted to 1/4000 in PBS/0.05% Tween/10 g/l BSA and incubate for 1 hour at 37°C.

Wash plates as described.

Add 100 µl/well OPD substrate and incubate at room temperature in the dark for 10-20min. Stop the reaction by the addition of 50 µl/well 25% v/v sulphuric acid and read the O.D. at 490nm.

Treatment of Results

A standard curve of known concentrations of mouse anti-human IgE antibody *vs* O.D. is constructed. Test supernatants will be considered positive for antibody if their O.D. value is greater than the mean of background plus three times the standard deviation of the background (mean ±3SD). The background O.D. value is calculated from wells in the absence of anti-human IgE

mAb. For those test samples considered to be positive for anti-human IgE Abs a concentration will be assigned with reference to the standard curve.

FcεRIα binding assay (Protein A plates)

Introduction

In this assay, a recombinant form of the ectodomain of the alpha chain of the high affinity receptor for IgE (alpha ectodomain) is utilised to bind chimaeric IgE. The carboxyl terminus of the alpha ectodomain is fused to a human IgG1 Fc sequence. This enables the recombinant molecule to be bound to protein A coated microtitre plates via the Fc region. Hence, the majority of the alpha ectodomain molecules should be available for binding ligand and provides a system for the analysis of IgE - receptor interactions. The format described below is aimed at detecting the (high affinity) receptor blocking activity of anti-IgE antibodies.

Materials

Materials	**Source (Product Code)**
Protein A coated plates	Pierce (15130EE)
Reagent reservoirs	Costar (4870)
Recombinant α-ecto-Ig Fusion protein	In house
Human/mouse chimaeric IgE	Serotec (MCA 333B)
Goat anti-mouse lambda chain HRPO linked antibody	Harlan (SBA 1060-05)
Pig serum	Serotec (C15SC)
Bovine serum albumin (fraction V)	Sigma (A-2153)
Phosphate buffered saline	Sigma (P-4417)
Tween-20	Sigma (P-1379)
Phosphate-citrate buffer with sodium perborate	Sigma (P-4922)
O-phenylene diamine (OPD) tablets	Sigma (P-7288)
25% v/v sulphuric acid	Fisons (H/0564/21)

Equipment

Equipment	**Supplier (Code)**
Multichannel pipette	Finnpipette or similar
Ultrawash Plate washer	Dynex Technologies
1296-001Delfia Plateshake	Wallac
MRX Plate reader	Dynatech

Method

ELISA protocol for detection of binding of IgE to the alpha chain ectodomain of the high affinity receptor

Coat protein A plates with 100µl/well α-ecto-Ig fusion protein diluted to 0.25µg/ml in blocking buffer (PBS/5% BSA/0.05% Tween-20). Incubate 1 hour at 37°C.

Dilute chimaeric IgE to 0.03125µg/ml in 10% pig serum. Dilute anti-IgE antibody to appropriate test concentration(s) in this IgE solution. Incubate 1 hour at room temperature.

Wash plates three times with PBS/0.05% Tween-20 using plate washer.

Add 100µl/well of IgE:anti-IgE solution (quadruplicates of each anti-IgE concentration are assayed). Incubate 1 hour at 37°C.

Wash plates three times with PBS/0.05% Tween-20 using plate washer.

Add 100µl/well of goat anti-mouse lambda chain HRPO conjugated antibody diluted 1:6000 dilution in blocking buffer. Incubate 1 hour at 37°C.

Wash plates three times with PBS/0.05% Tween-20 using plate washer.

Add 200µl/well of OPD substrate and incubate at room temperature in the dark for 2-10 minutes. Stop the reaction by the addition of 25µl 25% H_2SO_4. Mix stopped reactions on plateshaker - SLOW speed. Read OD at 490nm.

Treatment of Results

A figure for the percentage of inhibition of binding of IgE to its receptor can be calculated. A maximum binding value for IgE is determined from the average of a set of wells that contained IgE in 10% pig serum alone (i.e no anti-IgE).

The % inhibition value is calculated thus:
(max IgE value - average of anti-IgE replicates)/max IgE value x 100

FcεRIα binding assay (Clipped ectodomain)

This assay is essentially identical to the previous assay except that the ectodomain/IgG construct is treated with the proteolytic enzyme Factor X to cleave the two moieties. The IgG Fc moiety is removed using protein A beads, and the Factor X is removed using strepatavidin beads, thus leaving an essentially pure alpha chain ectodomain product. In this assay format, the alpha ectodomain is bound directly to plastic microtitre plates, all other assay details are as described above.

CD23-binding assay.

Method

Harvest, wash and re-suspend RPMI 8866 cells at 10^6/ml in sterile PBS. Add 500 µl to a 5ml FACS tube.

Add 500 µl chimeric human IgE (Serotec MCA333B) diluted in PBS at x2 the required final dilution and add to the cells. Incubate on ice for 1hr.

Note: Where blocking antibodies are to be tested the chimeric IgE is incubated with the blocking antibody for 1hr at room temperature prior to addition to the cells.

Wash cells twice by centrifugation at 270g for 5min in an excess of PBS.

Re-suspend cells in 500µl of PBS containing 10 µl of goat anti-human IgE antibody conjugated to FITC (Biosource AHI0508). Incubate on ice in dark for 1 hr.

Repeat wash step 4.3

Re-suspend cells in 500µl of PBS containing 5 µg/ml propidium iodide (Sigma). Briefly vortex to mix.

Collect and analyse 10,000 live gated events by flow cytometry

HBA Assay

Materials

Material	Source (Product Code)
Human blood	In-house from allergic donor with defined sensitivity to *Lol p* I
EDTA	BDH (100935V)
Ficoll-Paque	Pharmacia (17-0840-02)
HEPES buffered Hanks' balanced salt solution (HBH)	In-house (prepared according to Document No. srgt23
Human serum albumen (HSA)	Sigma (A8763)
Lol p I soluble extract	ALK UK (223204)
Immunotech histamine EIA kit	Serotec (2562)

Equipment

Equipment	Supplier (Code)
50 ml plastic disposable syringes	Becton Dickinson
19 or 21 gauge sterile hypodermic needles	Becton Dickinson
Blood collection tubes	Not critical
96-well V-bottom cell culture plates	Costar (3894)
Benchtop centrifuge capable of accepting 50ml tubes, and giving 500Xg	Not critical
MRX ELISA plate reader	Dynex Technologies

Method

Blood collection and cell preparation.

Blood is collected by venepuncture into tubes containing 0.1 volumes 2.7% EDTA, pH 7.0. It is then diluted 1/2 with an equal volume of HBH containing 0.1% HSA (HBH/HSA).

The resulting cell suspension is carefully layered over 50% volume Ficoll-Paque and centrifuged at 400g for 30 minutes at room temperature. The peripheral blood mononuclear cell (PBMC) layer at the interface is collected and the pellet is discarded.

The cells are washed once in HBH/HSA, counted, and re-suspended in HBH/HSA at a cell density of 2.0×10^6 per ml.

Cell Incubations

100µl cell suspension are added to wells of a V-bottom 96-well plate containing 100µl diluted test sample. Each test sample is tested at a range of dilutions with 6 wells for each dilution.

Well contents are mixed briefly using a plate shaker, before incubation at 37°C for 30 minutes with shaking at 120 rpm.

For each serum dilution 3 wells are triggered by addition of 10µl *Lol p* I extract (final dilution 1/10000) and 3 wells have 10µl HBH/HSA added for assessment of anaphylactogenicity.

Well contents are again mixed briefly using a plate shaker, before incubation at 37°C for a further 30 minutes with shaking at 120 rpm.

Incubations are terminated by centrifugation at 500g for 5 min. Supernatants are removed for histamine assay using the standard histamine method provided with the kit.

Control wells containing cells without test sample are routinely included to determine spontaneous and triggered release. Wells containing cells + 0.05% Igepal detergent are also included to determine total cell histamine.

Treatment of Results

Anaphylactogenesis assay

Histamine release due to test samples =

% histamine release from test sample treated cells - % spontaneous histamine release.

Blocking assay

The degree of inhibition of histamine release can be calculated using the formula:

$$\% \text{ inhibition} = 1 - \frac{\text{(histamine release from test sample treated cells*)}}{\text{(histamine release from antigen stimulated cells*)}} \times 100$$

* Values corrected for spontaneous release.

Protocol For the Panning of Phage Libraries Against Biotinylated Antibody

Day 1 (First Round Elution)

1. Take 500µl (5mg) of M280 streptavidin beads. Resuspend the beads in 1ml 4%MPBS (4%Marvel dried milk in PBS) and incubate on a rotating turntable at R.T. for 1hour. After blocking, pellet the beads (13000 rpm for 15secs) and resuspend in 0.5ml of 2%MPBS.

2. Dilute $5x10^{11}$ pfu of phage into 1.5ml of 2%MPBS. Incubate at R.T. for 30mins. Add 10µg of biotinylated antibody in a minimum volume. Place on a rotating turntable for 1hour at R.T.

3. Use 0.7ml of K91 cells to inoculate 11ml of NZY. Grow shaking at 225rpm, 37°C until OD_{600} = 1.8.

4. Add 0.5ml of blocked beads from step 1 to the mixture from step 2 and incubate on a rotating turntable for 30mins at R.T. Wash the beads using 3x1ml PBS-0.1%Tween and 3x1ml PBS.

5. Resuspend the pellet in 0.5ml of 0.1M HCl, 1mg/ml BSA, pH adjusted to 2.2 using glycine. Incubate at R.T. for 15mins,

pellet the beads, remove the supernatant **AND KEEP**. Neutralise using 1ml of 1M Tris pH8.5.

6. Wash the beads x3 using 1ml PBS and resuspend the pellet in 0.5ml of 0.1M TEA (triethyl acetate). Incubate at R.T. for 15mins, pellet the beads, remove the supernatant **AND KEEP**. Neutralise using 1ml of 1M Tris pH8.5.

7. When the cells have reached OD_{600} ~1.8 shake for 10mins at 50rpm and add half of the pooled, eluted phage. Leave at R.T. for 10mins with occasional swirling.

8. Add 90ml of NZY medium to the culture and 0.2µg/ml tetracycline. Grow for 30mins at 225rpm for 30mins.

9. Take 5µl of the culture and titer as plaques on NZY plates. Increase the tetracycline concentration of the culture to 15µg/ml.

10. Grow the culture overnight at 225rpm, 37°C.

Day 2 (First Round Amplification)

11. Spin the overnight culture at 10000rpm for 10minutes. Remove the supernatant carefully and add 0.15 volumes of 16.7%PEG/3.3M NaCl. Allow phage to precipitate for 2 hours at 4°C.

12. Spin both PEG precipitates at 15000rpm for 15mins. Remove the supernatant thoroughly and resuspend the pellet in 1ml PBS.

13. Centrifuge for 10mins at 13000rpm to pellet residual cells. Transfer the supernatant to a fresh tube and reprecipitate using 0.15 volumes of 16.7%PEG/3.3M NaCl for 1hour on ice.

14. Centrifuge at 13000rpm for 15mins and discard the supernatant. Resuspend the pellet in 200µl of PBS, 0.02% azide.

15. Titer the purified phage as plaques on NZY plates.

Day 3 (Second Round Elution)

1. Take 500µl (5mg) of M280 streptavidin beads. Resuspend the beads in 1ml 4%MPBS and incubate on a rotating turntable at R.T. for 1hour. After blocking, pellet the beads (13000 rpm for 15secs) and resuspend in 0.5ml of 2%MPBS.

2. Dilute $5x10^{11}$ pfu of phage into 1.5ml of 2%MPBS. Incubate at R.T. for 30mins. Add 10µg of biotinylated antibody in a minimum volume. Place on a rotating turntable for 1hour at R.T.

3. Use 0.7ml of K91 cells to inoculate 11ml of N2Y. Grow shaking at 225rpm, 37°C until OD_{600} = 1.8.

4. Add 0.5ml of blocked beads from step 1 to the mixture from step 2 and incubate on a rotating turntable for 30mins at R.T. Wash the beads using 3x1ml PBS-0.1%Tween and 3x1ml PBS.

5. Resuspend the pellet in 0.5ml of 0.1M HCl, 1mg/ml BSA, pH adjusted to 2.2 using glycine. Incubate at R.T. for 15mins, pellet the beads, remove the supernatant **AND KEEP**. Neutralise using 1ml of 1M Tris pH8.5.

6. Wash the beads x3 using 1ml PBS and resuspend the pellet in 0.5ml of 0.1M TEA. Incubate at R.T. for 15mins, pellet the beads, remove the supernatant **AND KEEP**. Neutralise using 1ml of 1M Tris pH8.5.

7. When the cells have reached OD_{600} ~1.8 shake for 10mins at 50rpm. Pour 10ml into a flask and add half of the pooled, eluted phage. Leave at R.T. for 10mins with occasional swirling.

8. Add 90ml of NZY medium to the culture and 0.2µg/ml tetracycline. Grow for 30mins at 225rpm for 30mins.

9. Take 5µl of the culture and titer as plaques on NZY plates. Increase the tetracycline concentration of the culture to 15µg/ml.

10. Grow the culture overnight at 225rpm, 37°C.

Day 4 (Second Round Amplification)

11. Spin the overnight culture at 10000rpm for 10minutes. Remove the supernatant carefully and add 0.15 volumes of 16.7%PEG/3.3M NaCl. Allow phage to precipitate for 2 hours at 4°C.

12. Spin both PEG precipitates at 15000rpm for 15mins. Remove the supernatant thoroughly and resuspend the pellet in 1ml PBS.

13. Centrifuge for 10mins at 13000rpm to pellet residual cells. Transfer the supernatant to a fresh tube and reprecipitate using 0.15 volumes of 16.7%PEG/3.3M NaCl for 1hour on ice.

14. Centrifuge at 13000rpm for 15mins and discard the supernatant. Resuspend the pellet in 200µl of PBS, 0.02% azide.

15. Titer the purified phage as plaques on NZY plates.

Day 5 (Third Round Elution)

1. Take 100µl (2mg) of M280 streptavidin beads. Resuspend the beads in 1ml 4%MPBS and incubate on a rotating turntable at R.T. for 1hour. After blocking, pellet the beads (13000 rpm for 15secs) and resuspend in 0.2ml of 2%MPBS.

2. Dilute $5x10^{11}$ pfu of phage into 1.5ml of 2%MPBS. Incubate at R.T. for 30mins. Add 5µg of biotinylated antibody in a minimum volume. Place on a rotating turntable for 1hour at R.T.

3. Use 0.7ml of K91 cells to inoculate 11ml of NZY. Grow shaking at 225rpm, 37°C until OD_{600} = 1.8.

4. Add 0.2ml of blocked beads from step 1 to the mixture from step 2 and incubate on a rotating turntable for 30mins at R.T. Wash the beads using 3x1ml PBS-0.1%Tween and 3x1ml PBS.

5. Resuspend the pellet in 0.5ml of 0.1M HCl, 1mg/ml BSA, pH adjusted to 2.2 using glycine. Incubate at R.T. for 15mins, pellet the beads, remove the supernatant **AND KEEP**. Neutralise using 1ml of 1M Tris pH8.5.

6. Wash the beads x3 using 1ml PBS and resuspend the pellet in 0.5ml of 0.1M TEA. Incubate at R.T. for 15mins, pellet the beads, remove the supernatant **AND KEEP**. Neutralise using 1ml of 1M Tris pH8.5.

7. When the cells have reached OD_{600} ~1.8 shake for 10mins at 50rpm. Pour 10ml into a flask and add half of the pooled, eluted phage. Leave at R.T. for 10mins with occasional swirling.

8. Add 90ml of NZY medium to the culture and 0.2µg/ml tetracycline. Grow for 30mins at 225rpm for 30mins.

9. Take 5µl of the culture and titer as plaques on NZY plates. Increase the tetracycline concentration of the culture to 15µg/ml.

10. Grow the culture overnight at 225rpm, 37°C.

Day 7 (Fourth Round Elution)

1. Take 100µl (1mg) of M280 streptavidin beads. Resuspend the beads in 1ml 4%MPBS and incubate on a rotating turntable at R.T. for 1hour. After blocking, pellet the beads (13000 rpm for 15secs) and resuspend in 0.1ml of 2%MPBS.

2. Take 75µl of phage from both the third round acid and base elutions and block with 1.5ml of 2%MPBS. Incubate at R.T. for 30mins. Add 1µg of biotinylated antibody in a minimum volume. Place on a rotating turntable for 1hour at R.T.

3. Add 0.1ml of blocked beads from step 1 to the mixture from step 2 and incubate on a rotating turntable for 30mins at R.T. Wash the beads using 3x1ml PBS-0.1%Tween and 3x1ml PBS.

4. Resuspend the pellet in 0.5ml of 0.1M HCl, 1mg/ml BSA, pH adjusted to 2.2 using glycine. Incubate at R.T. for 15mins, pellet the beads, remove the supernatant **AND KEEP**. Neutralise using 1ml of 1M Tris pH8.5.

5. Wash the beads x3 using PBS and resuspend the pellet in 0.5ml of 0.1M TEA. Incubate at R.T. for 15mins, pellet the beads, remove the supernatant **AND KEEP**. Neutralise using 1ml of 1M Tris pH8.5.

6. Take 10µl of the eluted phage and plate as plaques on NZY plates. Pick the plaques into NZY-TET for analysis.

Claims

1. A pharmaceutical composition for therapeutic/prophylactic treatment of IgE-mediated immune response such as allergy, comprising (A) an anti-IgE antibody, which recognises the epitope QVMDVDL [SEQ ID No 1] of the human IgE Cε2 Fc domain, together with (B) a pharmaceutically and physiologically acceptable carrier, diluent, excipient, adjuvant or the like.

2. A composition according to claim 1 wherein the antibody is the monoclonal antibody PTmAb0005.

3. An anti-allergy vaccine containing or consisting of a composition according to claim 1 or 2.

4. Use of antibody PTmAb0005 or an antibody as defined in Claim 1(A) in the manufacture of a medicament for treatment/prophylaxis of an IgE-mediated immune response such as allergy.

5. A method of treatment/prophylaxis of immune response such as allergy, which comprises administering to a patient an anti-allergy-effective amount of a composition according to claim 1 or 2.

6. A method of treatment/prophylaxis of immune response such as allergy, which comprises administering to a patient an anti-allergy-effective amount of a vaccine according to claim 3.

7. Use of anti-IgE antibody or an antibody as defined in Claim 1(A) in an assay to define therapeutically useful sequences from IgE for use as vaccines when coupled to a suitable carrier, such useful sequences being capable of eliciting an anti-IgE immune response in a vaccinated patient.

8. A method of assaying for a therapeutically useful sequence from IgE for use as a vaccine when coupl d to a suitable

carrier; which method includes the step of contacting a library of possible useful sequences of peptides with anti-IgE antibody or an antibody as defined in Claim 1(A)under antibody binding conditions; identifying one or more sequences which bind to the antibody, and testing said one or more sequences for immunogenicity in vivo.

9. A method according to claim 8 which further includes the step of synthesising an isolated pure polypeptide containing an immunogenic sequence identified by the testing step of the method.

10. A method according to claim 8 or 9 which includes the step of synthesising an isolated and purified anti-IgE immunogenic compound which consists of or includes partly or wholly non-peptidic mimetic of said one or more sequences identified by the testing step of the method.

11. A polypeptide or peptidomimetic produced by the method of claim 9 or 10.

12. A polypeptide have the sequence QVMDVDL (SEQ ID NO. 1).

13. A composition which comprises a polypeptide according to claim 12, together with a carrier and an adjuvant.

14. An immunogen comprising a peptide and a carrier molecule; characterised in that said immunogen is capable of inducing a humoral immune response which binds to the IgE peptide sequence QVMDVDL [SEQ ID No 1].

15. An anti-allergy vaccine comprising a composition according to claim 13, or an immunogen according to claim 14.

16. Use of a polypeptide according to claim 12, or an immunogen according to claim 14, in the manufacture of a

medicament for treatment/prophylaxis of an IgE-mediated immune response such as allergy.

17. A method of treatment/prophylaxis of immune response such as allergy, which comprises administering to a patient an anti-allergy-effective amount of a composition according to claim 13 or an immunogen according to claim 14.

18. A method of treatment/prophylaxis of immune response such as allergy, which comprises administering to a patient an anti-allergy-effective amount of a vaccine according to claim 15.

19. An anti-IgE antibody which recognises the epitope QVMDVDL [SEQ ID No 1] and has the following properties

i. It is capable of binding human IgE in its non-receptor-bound state (i.e. in free solution or when bound to a solid phase support e.g. an ELISA plate).

ii. It is capable of binding IgE bound to its high affinity receptor (FcεRI).

iii. It will prevent binding of IgE to the high affinity IgE receptor (FcεRI).

iv. It will not prevent IgE binding to the low affinity IgE receptor (FcεRII).

v. It inhibits degranulation of human blood basophils following exposure to antigen.

but excluding antibody PTmAb0005.

20. A pharmaceutical composition for therapeutic/prophylactic treatment of IgE-mediated immune response such as allergy, comprising (A) an anti-IgE antibody, which recognises the

epitope QVMDVDL [SEQ ID No 1] of the human IgE Cε2 Fc domain and which has the following properties:

i. It is capable of binding human IgE in its non-receptor-bound state (i.e. in free solution or when bound to a solid phase support e.g. an ELISA plate).

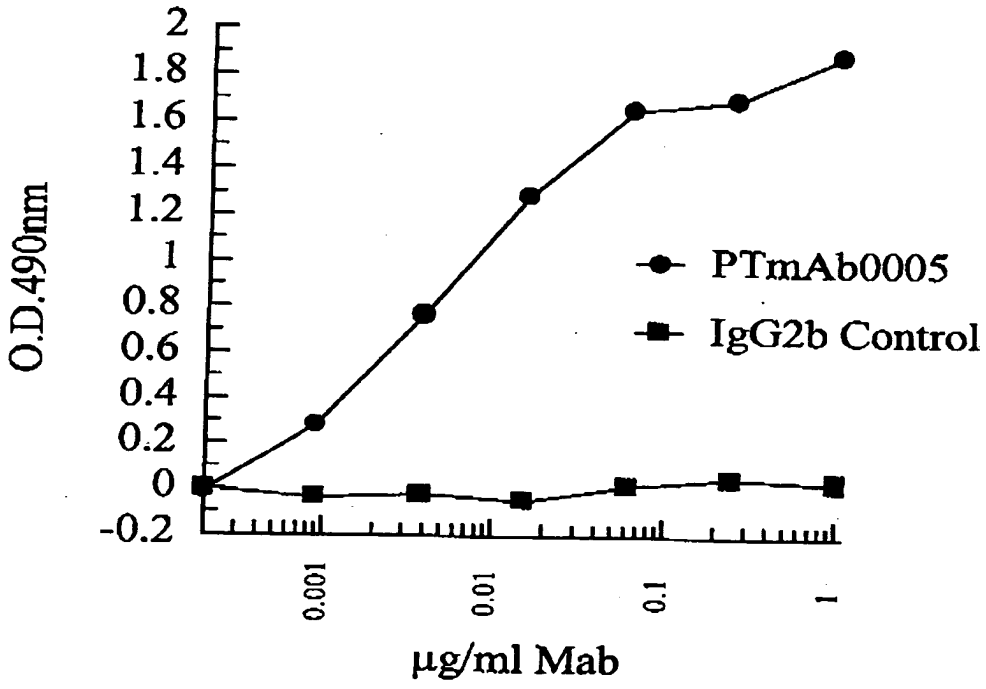
ii. It is capable of binding IgE bound to its high affinity receptor (FcεRI).

iii. It will prevent binding of IgE to the high affinity IgE receptor (FcεRI).

iv. It will not prevent IgE binding to the low affinity IgE receptor (FcεRII).

v. It inhibits degranulation of human blood basophils following exposure to antigen.

together with (B) a pharmaceutically and physiologically acceptable carrier, diluent, excipient, adjuvant or the like.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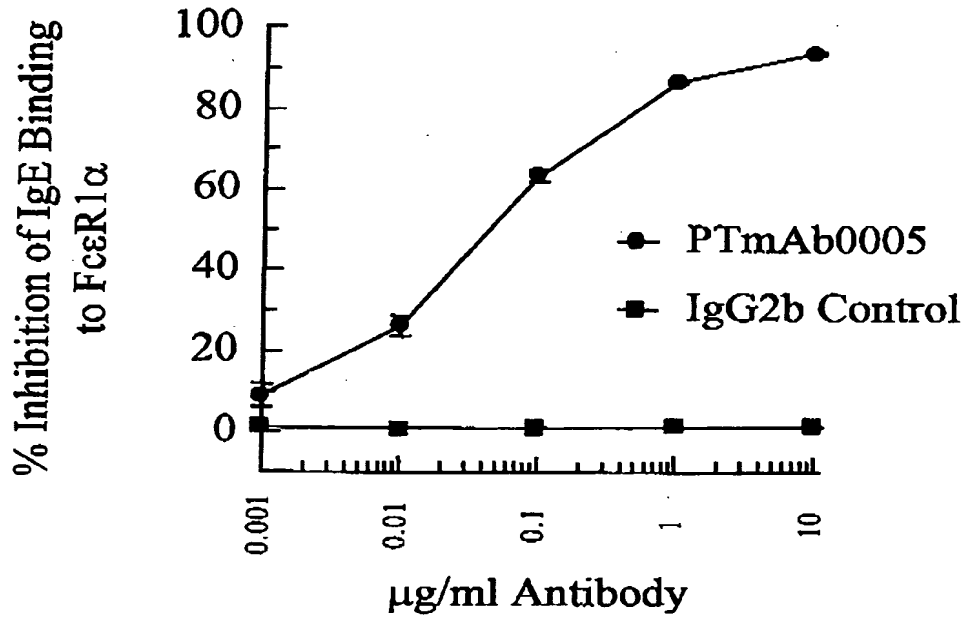
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
0.001
0.01
0.1
1
µg/ml Mab
PTmAb0005
IgG2b Control

Fig.1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100
80
60
40
20
0
0.001
0.01
0.1
1
10
µg/ml Antibody
PTmAb0005
IgG2b Control

Fig.2

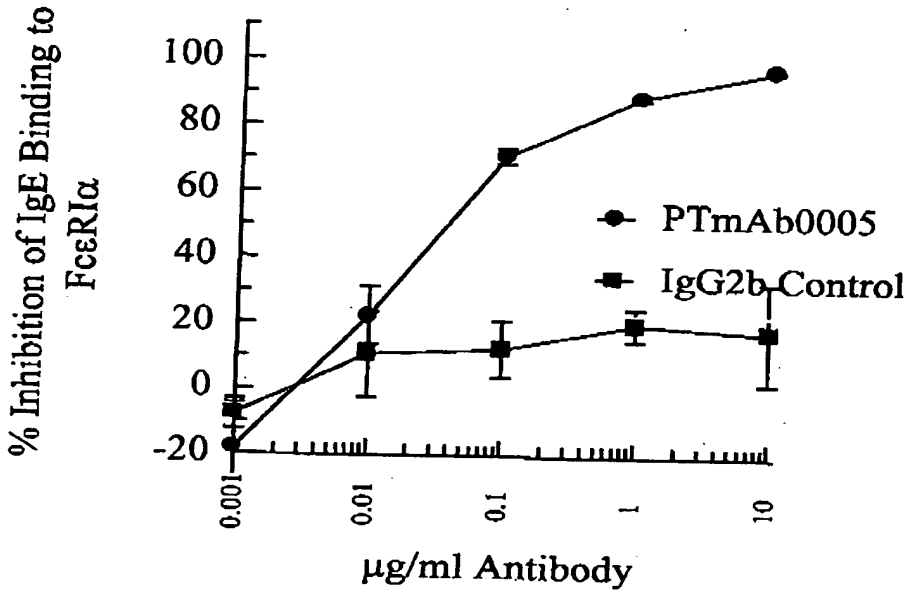

% Inhibition of IgE Binding to FcεRIα
100
80
60
40
20
0
-20
0.001
0.01
0.1
1
10
µg/ml Antibody
PTmAb0005
IgG2b Control

Fig.3

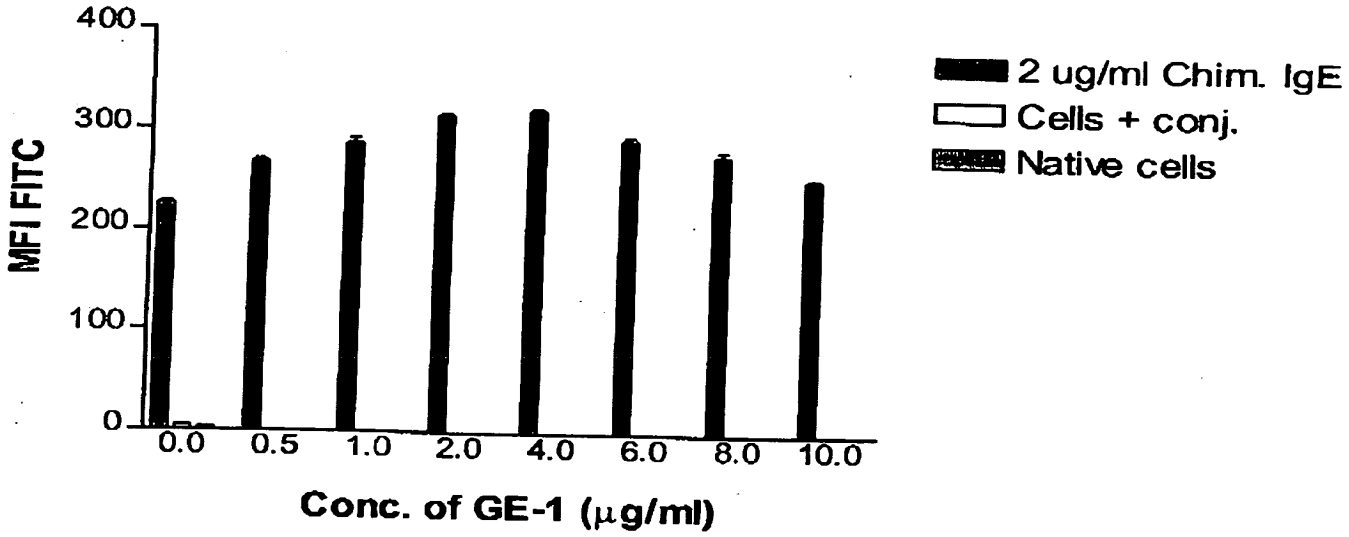
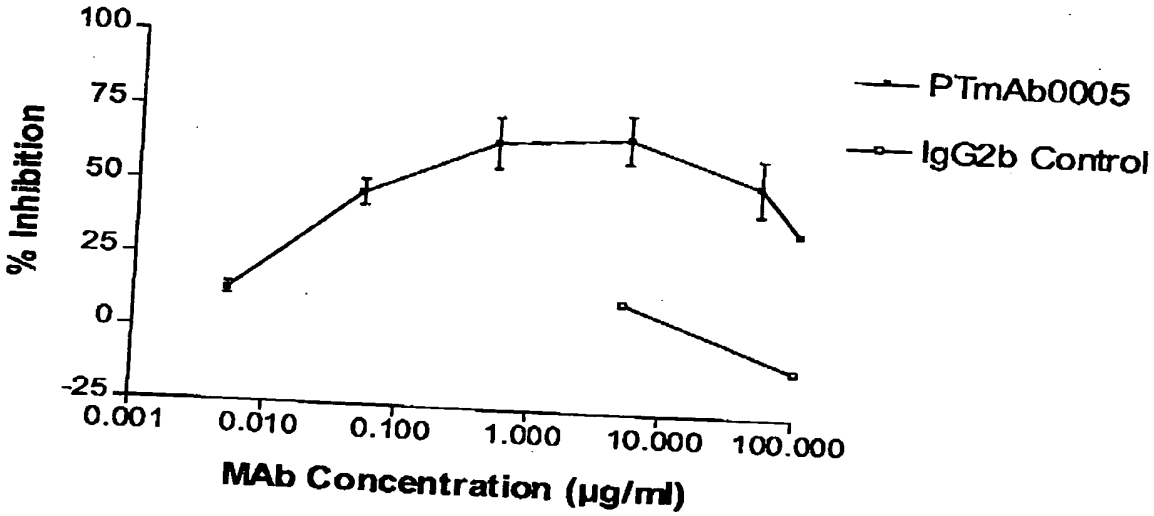

400
300
200
100
0
MFI FITC
0.0
0.5
1.0
2.0
4.0
6.0
8.0
10.0
Conc. of GE-1 (µg/ml)
2 ug/ml Chim. IgE
Cells + conj.
Native cells

Fig. 4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75
50
25
0
-25
% Inhibition
0.001
0.010
0.100
1.000
10.000
100.000
MAb Concentration (µg/ml)
PTmAb0005
IgG2b Control

Fig.5